Remarks

The present invention relates to intracellular receptors, methods for the modulation thereof, and methods for the identification of novel ligands therefor. In a particular aspect, the present invention relates to members of a family of silencing mediators of retinoic acid and thyroid hormone receptors (SMRT co-repressors). Exemplary members of the SMRT co-repressor family include various isoforms of human, mouse and Drosophila SMRT co-repressor polypeptides that are capable of mediating the transcriptional silencing of at least one member of the steroid/thyroid hormone superfamily of receptors.

Claims 1-37 were pending before this communication. Claims 26-37 have been withdrawn from consideration pursuant to the election of Group I (claims 1-25) with traverse. By this response, the specification and claims 1, 3, 5, 6, 9, 10, 12, 14, 15 and 23 have been amended to define Applicants' invention with greater particularity. These amendments add no new matter as they are fully supported by the specification and the original claims. Claim 2 has been cancelled without prejudice. Attached hereto is a marked-up version of the changes made to the specification and claims, labeled APPENDIX A.

Accordingly, claims 1 and 3-25 are currently under consideration. For the Examiner's convenience, a clean copy of these claims is also provided in APPENDIX B. Claims 1 and 3-37 remain pending in this application.

Applicants acknowledge the Examiner's request for compliance with the requirements of 37 C.F.R. § 1.821-1.825. The specification has been amended to designate SEQ ID NOs consistent with their usage in the sequence listing, and to add SEQ ID NOs for previously unnumbered sequences. In particular, on page 66, line 19, the specification has been amended to designate a new SEQ ID NO:13 for the nine amino acid nuclear targeting signal. In addition, on page 66 in various locations, SEQ ID NO:1 has been replaced with reference to the SMRTER sequence SEQ ID NO:12 used in Example 24. On pages 4-8, various figure legends have been amended to identify sequences contained in the respective figures by appropriate corresponding

SEQ ID NOs. In order to incorporate unidentified sequences in the specification and figures that are not present in the current sequence listing, a new sequence listing is being submitted concurrently to BOX SEQUENCE, and a copy of the paper Sequence Listing is enclosed herewith.

Applicants acknowledge that the signature of Inventor Chen is missing from the filed declaration. The defective declaration is being addressed under separate cover.

The Examiner's objection to the drawings because Figures 4, 5A, 6C, 9 and 12C are allegedly illegible is acknowledged. Responsive to this objection, replacement Figures 4, 5, 6, 9 and 12 are enclosed herewith. Accordingly, Applicants respectfully request reconsideration and withdrawal of this objection to the drawings.

Responsive to the objections to the disclosure because of several alleged informalities, the specification has been amended as follows:

On page 1, the specification has been amended to add the Serial Number of the related pending application.

On page 4, line 31, the specification has been amended to add the GenBank accession number.

On page 43, line 10, the specification has been amended to add the definition for the abbreviation "PML" (promyelocytic leukemia).

On page 5, line 9, the specification has been amended to replace reference to "Figure 3" with the phrase "Figures 3A-3D" to more clearly reflect the content of the figure.

On page 42, line 15, the specification has been amended to refer to the carboxy-terminal transactivation domain only as AF-2, as it is known in the art.

On page 50, lines 2 and 18, the specification has been amended to correct a typographical error so as to refer to SEQ ID NO:4, rather than SEQ ID NO:3.

On page 50, lines 11-12, the specification has been amended to refer to SEQ ID NOs:8 and 9 in connection with the splice variant of SEQ ID NO:7.

On page 49, line 24, the 3.5 kilobase size refers to a cDNA fragment that was an intermediate in obtaining the final full-length human SMRT (SEQ ID NO:4), corresponding to a 5.3 kilobase cDNA. On page 49, line 31, the specification has been amended to properly refer to SEQ ID NO:4 as the full-length human SMRT nucleotide sequence.

Throughout pages 43-49, the specification has been amended to replace references to the "full-length SMRT" with the "first SMRT" cDNA, clone or sequence cloned. This clarifies that the present invention encompasses the longer full-length clone subsequently obtained in Example 9.

On page 55, line 19, the specification has been amended to add the definition for the abbreviation "EcR" (ecdysone receptor).

On page 76, the Abstract has been amended to reduce the length to less than 150 words.

Accordingly, Applicants respectfully request reconsideration and withdrawal of these objections to the disclosure.

Responsive to the objections to claim 1 because SMRT is not defined at its first use in the claim set, claim 1 has been amended to incorporate "SMRT" after the full length descriptor "silencing mediators of retinoic acid receptor and thyroid hormone receptor" (emphasis added). Accordingly, Applicants respectfully request reconsideration and withdrawal of this objection to claim 1.

The objection to claim 2 under 37 CFR § 1.75(c) as allegedly being of improper dependent form for failing to further limit the subject matter of a previous claim is respectfully traversed. However, in order to reduce the issues and expedite prosecution, claim 2 has been cancelled and the description of the SMRT co-repressors of the present invention has been added to claim 1 to further define Applicants' invention with greater particularity. Accordingly, the objection to claim 2 is rendered moot by the present amendment.

The provisional rejection of claims 1-7, 19, 21 and 22 under the judicially created doctrine of obviousness-type double patenting as being allegedly unpatentable over claims 6 and 7 of copending Application No. 08/522,726 is respectfully traversed. Appellants respectfully submit that the claims are patentably distinct from each other because claims 6 and 7 of 08/522,726 (as allowed) require additional features which are not required by claims 1-7, 19, 21 and 22 of the present application. However, in order to reduce the issues and expedite prosecution, Appellants are submitting herewith a terminal disclaimer disclaiming any term extending beyond the expiration date of 08/522,726 upon grant according to the enclosed terminal disclaimer. Accordingly, Applicants respectfully request reconsideration and withdrawal of this provisional rejection.

The rejection of claims 1-3, 5, 6, 8, 11 and 19-22 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey that Applicants had possession of the claimed invention is respectfully traversed. Applicants respectfully submit that several exemplary members of the genus have been clearly described, providing structure and function for all members of the genus. The members share extensive regions of homology as described below, and all function as co-repressors which interact with well-known transcriptional mediators (i.e., members of the steroid/thyroid hormone superfamily of receptors).

The family of silencing mediators consists of SMRT co-repressors that share structural homology across distinct regions of their N-terminal domains. As noted by the Examiner, the N-terminal domain is responsible for the repression activity of the protein (see Office Action, Paper No. 10, at page 8, lines 5-8). Therefore, the structural homology is related to the conserved co-repression function of all family members.

Specific examples provided in the specification show that the RD1 region (amino acids 1-312) and the SANT region (amino acids 312-668) both within the N-terminal domain of hSMRT co-repressor. share substantial sequence identity with the corepressor hN-CoR. Within the RD1 and SANT regions themselves, there are specific regions of even higher identity over shorter

amino acid stretches (see, for example, Figure 5B). Moreover, the human and mouse alpha SMRT members are virtually identical across their entire lengths. The conserved regions of identity within the N-terminal domain provide the regions that are related to the conserved co-repression function exhibited by all members of the family. Thus, these regions within the N-terminal clearly describe the members of the genus encompassed by claims 1, 3, 5, 6, 8, 11 and 19-22, such that one of skill in the art would have no reason to doubt that Applicants had possession of the genus at the time of filing. Accordingly, Applicants request reconsideration and withdrawal of this rejection of claims 1, 3, 5, 6, 8, 11 and 19-22.

The rejection of claims 4, 7, 9, 10 and 12 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey that Applicants had possession of the claimed invention is respectfully traversed. Applicants respectfully submit that each of these claims makes explicit reference to one SEQ ID NO. which is completely described by its entire sequence, and conservative variations thereof. Such terminology would readily be understood by one skilled in the art.

Applicants respectfully disagree with the Examiner's assertion that "one of skill in the art cannot envision all the conservative variations" of the respective SEQ ID NOs (see Office Action, Paper No. 10, page 9, lines 14-15). The term "conservative variations" is commonly used in the art to represent the substitution of codons encoding similar amino acid moieties such that the tertiary structure of the protein is not substantially altered (see specification at page 18, lines 12-14). The specification provides exemplary conservative amino acid variations, such as substitution of a non-polar residue for another non-polar residue or a charged residue for a similarly charged residue (see specification at page 18, lines 9-12). The Examiner has provided no evidence or reasoning as to why one of skill in the art would not recognize the standard use of the term "conservative variations" to clearly identify the structure of all claimed variations of each SEQ ID NO provided. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection of claims 4, 7, 9, 10 and 12.

The rejection of claims 1-3, 5, 6, 8, 11, 16 and 19-22 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains to use the invention commensurate in scope with the claims, is respectfully traversed. Appellants respectfully submit that the claims, as amended, are enabling for any of the polynucleotides claimed. The SMRT co-repressor encoded by the claimed polynucleotide is described by structure (see, for example, specification at page 9, lines 4-13) and by function (i.e., as capable of mediating the transcriptional silencing of at least one member of the steroid/thyroid hormone superfamily of receptors).

As acknowledged by the Examiner, the specification is "enabling for a polynucleotide sequence comprising SEQ ID NOs: 4, 6, 8 or sequence variants of sub-sequences of these or sequences which hybridize to one of these and have co-repression activity for retinoic acid receptor (RAR) and thyroid hormone receptor (TR)" (see Office Action, Paper No. 10, at page 9, line 22 through page 10, line 3). Appellants have amended claim 1 to embrace only those polynucleotides which encode a SMRT member "that is capable of mediating the transcriptional silencing of at least one member of the steroid/thyroid hormone superfamily of receptors". Claims 3, 5, 6, 8, 11, 16 and 19-22 as amended all ultimately depend on claim 1. Thus, the amended claims embrace only those polynucleotides that possess co-repression activity for at least one member of the steroid/thyroid hormone superfamily of receptors. It is respectfully submitted that Applicants have clearly set forth how to make and use the present invention as required by 35 U.S.C. § 112, first paragraph, and that all claims as amended are enabled by the specification as filed. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection of claims 1-3, 5, 6, 8, 11, 16 and 19-22.

The rejection of claims 4, 7, 9, 10 and 12 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains to use the invention commensurate in scope with the claims, is respectfully traversed. For all of the reasons noted above, Appellants respectfully submit that the claims, as amended, are enabling for any polynucleotides as claimed,

wherein the SMRT co-repressor encoded by the polynucleotide is capable of mediating the transcriptional silencing of at least one member of the steroid/thyroid hormone superfamily of receptors.

Applicants are claiming only those polynucleotides that possess co-repression activity. It is respectfully submitted that Applicants have clearly set forth how to make and use the present invention as required by 35 U.S.C. § 112, first paragraph, and that all claims as amended are enabled by the specification as filed. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection of claims 4, 7, 9, 10 and 12.

The rejection of claims 1-25 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite is respectfully traversed. Applicants respectfully submit that each claim as amended particularly points out and distinctly claims the subject matter which Applicants regard as their invention.

With respect to claim 1, Applicants respectfully disagree with the Examiner's assertion that "there is no art-recognized definition of what is considered to be an isoform" (see Office Action, Paper No. 10, at page 14, lines 17-19). It is respectfully submitted that the term "isoform" is used consistent with its dictionary definition, i.e., a protein having the same function and similar sequence (The Dictionary of Cell Biology, p.122, J.M. Lackie and J.A.T. Dow, eds, Academic Press, Ltd., 1989). Those of skill in the art would readily recognize what is encompassed by the term as employed in the claim.

With respect to claim 2, the rejection has been rendered moot as this claim has been cancelled.

With respect to claims 3, 5 and 15, Applicants respectfully submit that the phrase "hybridizes under stringent conditions" is so commonly accepted in the art that it no longer requires definition for one of skill in the art to immediately recognize what is meant by the phrase as employed in relation to the claimed polynucleotide sequence.

With respect to claims 4, 7, 9 and 12, Applicants respectfully submit that the phrase "conservative variations thereof" is so commonly accepted in the art that it no longer requires definition for one of skill in the art to immediately recognize what is meant by the phrase as employed in relation to the claimed sequences. Furthermore, as noted above, the specification provides exemplary substitutions that constitute conservative variations of a sequence (see, for example, specification at page 18, lines 10-15).

With respect to claims 9 and 12, the claim language has been amended to reflect that the claimed polynucleotides encode the respective amino acid sequences, as was clearly originally intended.

With respect to claim 10, the claim language has been amended to correct a typographical error and change the dependency to claim 8 which is drawn to a mouse SMRT co-repressor.

With respect to claim 15, the claim language has been amended to correct an obvious typographical error and insert the verb "hybridizes".

With respect to claim 23, the claim language has been amended to further clarify that the isolated oligonucleotide should not hybridize to either SEQ ID NO:11 or amino acids 1031-2517 of SEQ ID NO:5, as was clearly originally intended. Thus, the claim as amended clearly avoids the possibility of the erroneous interpretation that an alternative oligonucleotide is being claimed.

Therefore, each claim as amended particularly points out and distinctly claims the subject matter which Applicants regard as their invention. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 1-25 under 35 U.S.C. § 112, second paragraph.

Conclusion

In view of the above amendments and remarks, reconsideration and favorable action on all claims are respectfully requested. In the event any matters remain to be resolved in view of this communication, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

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Enclosures **Appendices A and B**
Five (5) sheets of drawings for replacement Figures 4, 5, 6, 9 and 12
Terminal Disclaimer
Paper copy of Sequence Listing

APPENDIX A – ALTERED SPECIFICATION AND CLAIMS VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

The paragraph beginning at line 7 of page 1 has been amended as follows:

-- This application is a continuation-in-part **[~~application~~]** of **[~~pending~~]** United States application Serial No. 08/522,726, filed September 1, 1995, **now pending,** and is related to United States application Serial No. **09/523,068[~~_____~~]**, filed on even date herewith, **now pending,** each of which is incorporated **by reference** herein in its entirety **[~~by reference~~]**. --

The paragraph beginning at line 30 of page 4 has been amended as follows:

-- Figure 2 presents amino acid (aa) sequences of **the first** SMRT **clone** (Genbank accession number **U37146[~~XXXXX~~]; SEQ ID NO:1**). The aa sequence presented in parentheses (i.e., residues 1330-1376) is an alternatively spliced insert which is not present in the original two-hybrid clone (C-SMRT, aa 981 to C-terminal end). The proline-rich N-terminal domain (aa 1-160) and the glutamine-rich region (aa 1061-1132), as well as the ERDR and SG regions, are also indicated. The C-terminal region of SMRT (aa 1201 to C-terminal end) shows 48% aa identity to RIP13 (Seol et al., *Molecular Endocrinology* 9:72-85 (1995)). The rest of the sequence of RIP13 shows 22% aa identity to SMRT (aa 819-1200). --

The paragraph beginning at line 9 of page 5 has been amended as follows:

-- **Figures 3A-3D illustrate** [~~**Figure 3 illustrates**~~] mediation of the silencing effect of hRARα and hTRβ by SMRT *in vivo*. --

The paragraph beginning at line 4 of page 6 has been amended as follows:

-- Figures 5A and 5B provide alignments of the human SMRT **(SEQ ID NO:5)** and human N-CoR **(SEQ ID NO:11)** co-repressors. --

The paragraph beginning at line 20 of page 6 has been amended as follows:

-- Figure 6C shows alignment of EcR **(amino acids 460-510; SEQ ID NO:14)**, rTR **(amino acids 215-265; SEQ ID NO:15)**, hRAR **(amino acids 227-277; SEQ ID NO:16)**, and rRev-erbA **(amino acids 401-451; SEQ ID NO:17)** receptor sequences and the secondary structure in the LBD signature motif region. Conserved residues are marked **with asterisks** [~~**in dark.**~~] The mutation 483 (AT) is marked at the top of the corresponding residue. --

The paragraph beginning at line 16 of page 7 has been amended as follows:

-- Figure 9 **shows sequence comparisons** [~~**. Sequence Comparison**~~] of SMRTER **(SEQ ID NOs:18, 21, 32, 35, 38 and 39)**, SMRT **(SEQ ID NOs:20, 23, 28, 33, 36 and 40-44)**, N-CoR **(SEQ ID NOs:19, 22, 27, 34, 37 and 45-51)**, and **other related proteins (SEQ ID NOs:24-26, 29-31 and 52)** [~~**Other Related Proteins**~~]. The SANT domains of various proteins are listed **(SEQ ID NOs:21-31)**. **Percentages of identity/similarity** [~~**Percent identities/similarities**~~] compared to SMRTER are shown on the right. Two potential helices are predicted in the N-terminal half of the SANT

domain. Black boxes indicate identical sequences; gray boxes **indicate** [,] similar or partially identical sequences. --

The paragraph beginning at line 16 of page 8 has been amended as follows:

-- Figure 12C shows an alignment of SMRD3 of SMRTER **(amino acids 2564-2588 of SEQ ID NO:12)** and an mSin3-interacting domain of N-CoR **(amino acids 1835-1859 of SEQ ID NO:11)**. Conserved residues are boxed **[~~in gray~~]**. An asterisk indicates the region where the mutation (Gly) was generated. Minus signs indicate that the interaction between SMRD3 and Sin3A was not detectable in the yeast two-hybrid assays. Repression was measured by comparing the transcriptional activity of Gal4-SMRD3 M2 or Gal4-SMRD3 M3 to that of wild-type Gal4-SMRD3 using transfection experiments as described above. --

The paragraph beginning at line 4 of page 42 has been amended as follows:

-- The function of SMRT as a silencing mediator (co-repressor) of RAR and TR is analogous to mSin3 in the Mad-Max-Sin3 ternary complex (Schreiber-Agus et al., *Cell* **80**:777-786 (1995); and Ayer et al., *Cell* **80**:767-776 (1995)). Because GAL-SMRT functions as a potent repressor when bound to DNA, it is reasonable to speculate that the function of the unliganded receptors is to bring with them SMRT to the template via protein-protein interaction. Thus, the repressor function is intrinsic to SMRT as opposed to the TR or RAR itself (Baniahmad et al., *Proc. Natl. Acad. Sci. USA* **90**:8832-8836 (1993); and Fondell et al., *Genes Dev* **7**:1400-1410 (1993)). It is demonstrated herein that the ligand triggers a dissociation of SMRT from the receptor, which would lead to an initial step in the activation process. This would be followed (or be coincident) with an induced conformational change in the carboxy-terminal transactivation domain (**known as** **[~~c, also called~~]** AF-2), allowing association with co-activators on the transcription machinery

(Douarin et al., *EMBO J.* 14:2020-2033 (1995); Halachmi et al., *Science* **264**:1455-1458 (1994); Lee et al., *Nature* **374**:91-94 (1995); and Cavailles et al., *Proc. Natl. Acad. Sci. USA* **91**:10009-10013 (1994)). Thus, as has previously been suggested (Damm and Evans, (1993), *supra*), the ligand dependent activation of TR would represent two separable processes including relief of repression and net activation. The isolation of SMRT now provides a basis for dissecting the molecular basis of trans-repression. --

The paragraph beginning at line 9 of page 43 has been amended as follows:

-- Total bacteria extracts expressing GST fusions of hRARα (aa 156-462) or hRXRα LBD (aa 228-462) and control extracts expressing GST alone or GST-PML **(promyelocytic leukemia)** fusion protein were subjected to SDS/PAGE and electroblotted onto nitrocellulose in transfer buffer (25 mM Tris, pH 8.3/ 192 mM glycine/ 0.01% SDS). After denaturation/renaturation from 6 M to 0.187 M guanidine hydrochloride in HB buffer (25 mM HEPES, pH 7.7/25 mM NaCl/5 mM $MgCl_2$/1 mM DTT) filters were saturated at 4°C in blocking buffer (5% milk, then 1% milk in HB buffer plus 0.05% NP40). *In vitro* translated ^{35}S-labeled proteins were diluted into H buffer (20 mM Hepes, pH 7.7/75 mM KCl/0.1 mM EDTA/2.5 mM $MgCl_2$/0.05% NP40/ 1% milk/1 mM DTT) and the filters were hybridized overnight at 4°C with (1 μM) or without ligand. After three washes with H buffer, filters were dried and exposed for autoradiography or quantitated by phosphoimager. --

The paragraph beginning at line 25 of page 43 has been amended as follows:

-- For yeast two-hybrid screening, a construct expressing the GAL4 DBD-hRXRα LBD (aa 198-462) fusion protein was used to screen a human lymphocyte cDNA library as described (Durfee et al., (1993), *supra*). **The first** ~~**[Full length]**~~ SMRT cDNA **(SEQ ID NO:1)** was isolated from a human HeLa cDNA library (Clontech) using the two-hybrid insert as a probe. --

The paragraph beginning at line 16 of page 44 has been amended as follows:

-- **The first** **[~~Full length~~]** SMRT **sequence cloned** encodes a polypeptide of 1495 amino acids rich in proline and serine residues (see Figure 2 and SEQ ID NO:1). Genbank database comparison reveals similarity of the C-terminal domain of SMRT to a partial cDNA encoding another receptor interacting protein, RIP13 (Seol et al., (1995), *supra*), whose role in receptor signaling is unknown. Within this region, there can be identified several potential heptad repeats which might mediate protein-protein interaction with the "α**[a]**-helical sandwich" structure (Bourguet et al., *Nature* 375:377-382 (1995)) of the ligand binding domain (LBD) of receptors. --

The paragraph beginning at line 18 of page 48 has been amended as follows:

-- In principle, over expression of SMRT should restore repressor activity when co-expressed with v-erbA or RAR403 competitors. Indeed, results presented in Figure 3C show that both the **whole first SMRT clone (SEQ ID NO:1)** **[~~full length~~]** and **its** **[~~the~~]** C-terminal domain of SMRT (C-SMRT) can titrate out v-erbA or RAR403 competitor activity and re-endow GAL-RAR and GAL-TR with silencing activity. In contrast, neither v-erbA nor SMRT show any effect on the transactivation activity of GAL-VP16 fusion. Thus, SMRT is able to block the titration effect of v-erbA and RAR403 and functionally replaces the putative SMRT co-repressor in this system. --

The paragraph beginning at line 30 of page 48 has been amended as follows:

-- If SMRT is the mediator of transcription silencing of TR and RAR by interaction with template-bound unliganded receptors, then direct recruitment of SMRT to a heterologous promoter should result in repression of basal level activity. This was tested by fusing **the whole first** ~~[full-length]~~ SMRT **clone (SEQ ID NO:1)** to the GAL4 DBD (GAL-SMRT). The effect of the resulting fusion protein on the activity of the thymidine kinase promoter containing four GAL4 binding sites was analyzed. Figure 3D shows that GAL-SMRT, like GAL-TR, can silence basal promoter activity in a dose-dependent manner. In contrast, GAL-RXR shows no repression. --

The paragraph beginning at line 19 of page 49 has been amended as follows:

-- An examination of the previously described human SMRT co-repressor revealed that the first eight amino acids and upstream sequences were derived from a portion of ribonucleoprotein K sequence. Accordingly, a mouse spleen cDNA lambda ZAP II library (Stratagene; La Jolla CA) was screened at low stringency with a probe corresponding to **the** approximately ~~[the 5']~~ 1,000 **5'** base pairs (bp) of the previously identified human SMRT (s-SMRT**; SEQ ID NO:1**). A 3.5 kilobase (kb) cDNA fragment was obtained that contained a unique sequence in addition to known s-SMRT sequence. The 5' end of this cDNA, and subsequently obtained clones, was used in successive rounds of screening of the mouse spleen cDNA library and a mouse brain cDNA library (Stratagene) and the full-length SMRTα isoform cDNA (SEQ ID NO: 6) and SMRT**β**~~[α]~~ isoform cDNA (SEQ ID NO: **8**~~[10]~~) were obtained. The mouse SMRT (m-SMRT) 5' sequence then was used at low stringency to screen a human pituitary cDNA library (Stratagene) to obtain the full-length human SMRT (h-SMRT) cDNA (SEQ ID NO:**4**~~[1]~~). All cDNA clones were sequenced on both strands using standard methods, and have been deposited with GenBank as Accession No. AF11~~[0]~~3003 (h-SMRT; SEQ ID NOS: 4~~[3]~~ and 5); Accession No. **AF**113001 (m-SMRTα ; SEQ ID NOS: 6 and 7); and Accession No. **AF**113002 (m-SMRTβ ; SEQ ID NOS: 8 and 9). --

The paragraph beginning at line 6 of page 50 has been amended as follows:

-- By sequentially shifting between the mouse spleen and mouse brain cDNA libraries, several clones containing a potential starting methionine and 5' untranslated region sequences were obtained. The complete polypeptide sequences of m-SMRT (SEQ ID NO: 7) and h-SMRT (SEQ ID NO: 5) are provided. In addition, a splice variant isolated from the mouse brain cDNA library **(SEQ ID NO:8)** encoded an m-SMRT co-repressor **(SEQ ID NO:9)** containing a deletion of amino acids 36 to 254 of SEQ ID NO: 7 ~~[(see SEQ ID NO: 3)]~~. The two m-SMRT co-repressors are designated SMRTα (SEQ ID NO: 7) and SMRTβ (SEQ ID NO: 9). Based on sequence similarity to N-CoR (see below), this deletion in m-SMRTβ removes the majority of the sequence in h-SMRT and m-SMRTα that is homologous to N-CoR repression domain 1 (RD1), including a portion of the Sin3A binding region. --

The paragraph beginning at line 18 of page 50 has been amended as follows:

-- The cloned h-SMRT (SEQ ID NO: **4**~~[3]~~) encodes a polypeptide that contains an additional 1130 amino acids at the amino terminus as compared to the previously described human SMRT co-repressor. The full length h-SMRT shares 84% identity with m-SMRTα. A comparison of h-SMRT (SEQ ID NO: 5) and N-CoR (SEQ ID NO: 11) revealed that the N-terminal extension of h-SMRT (amino acids 1 to 1030) and N-CoR (amino acids 1 to 1031) share approximately 41% identity, which is somewhat higher that the 36% identity shared between the full length proteins. However, regions within the N-CoR and SMRT N-termini share striking homology (Figures 4A and 4B). --

The paragraph beginning at line 19 of page 55 has been amended as follows:

-- To investigate whether repression by **the ecdysone receptor** (EcR) in CV-1 cells is mediated by its association with a vertebrate corepressor and whether such an interaction, if it does occur, is impaired by the A483T mutation, a mammalian two-hybrid assay with Gal4-c-SMRT was conducted. --

The paragraph beginning at line 1 of page 66 has been amended as follows:

-- In vitro pull down assays (Example 12) were conducted to determine whether EcR interacts with ERID1 and ERID2. In vitro translated 35S-methionine-labeled EcRB1 alone, or a mixture of 35S-methionine-labeled EcRB1 and unlabeled USP, or 35S-methionine-labeled USP alone, were incubated with GST, GST-ERID1 (amino acids 1698-2063 of SEQ ID NO:12[~~1~~]), or GST-ERID2 (amino acids **2929-3038** of SEQ ID NO:12[~~1~~]). GST-ERID1 and GST-ERID2, but not GST alone, pull down labeled EcR, whereas little interaction is found between USP and any of the three GST proteins. In addition, the pull-down complex was disrupted by the addition of 3μM MurA when USP is present. These in vitro results establish that SMRTER and EcR may interact directly. --

The paragraph beginning at line 12 of page 66 has been amended as follows:

-- Further in vitro tests were conducted to determine **if** ERID1, ERID2, and c-SMRT compete with each other to bind EcR. Gal4-ERID1 (amino acids 1698-2063 of SEQ ID NO:12[~~1~~]) or Gal4-ERID2 (amino acids 2929-3181 of SEQ ID NO:12[~~1~~]), along with EcR-vp16 and USP, were transfected in CV-1 cells as described above. In this competition experiment, additional ERID1, ERID2, and c-SMRT (Chen et al., (1996), *supra*) were cotransfected into cells. ERID1 (**amino acids** 1698-2063 **of SEQ ID NO:12**) and ERID2 [~~(~~](amino acids 2929-3038 of SEQ ID NO:12[~~1~~]) were tagged with the nuclear targeting signal (MAPKKKRKV) (SEQ ID NO:13[~~3~~]) to ensure that these proteins were localized in nuclei. As shown in Figure 11C, interaction between each

Gal4-ERID fusion and EcR-vp16:USP was significantly decreased by both ERIDs and by c-SMRT. Interestingly, a more prominent effect was observed in experiments when Gal4-ERID1 (amino acids 1698-2063 of SEQ ID NO:12[~~1~~]) was challenged by ERID2, and, conversely, a more efficient competition was achieved by ERID1 to Gal4-ERID2 (amino acids 2094-3181 of SEQ ID NO:12[~~1~~]). Together, these results suggest that ERID1, ERID2, and c-SMRT may bind similar or overlapping surface(s) in EcR. --

The Abstract paragraph on page 76 has been amended as follows:

-- The present invention relates to isolated polynucleotides encoding a family of silencing mediators of retinoic acid and thyroid hormone receptor (SMRT) isoforms, including vertebrate and invertebrate isoforms thereof. [~~For example, a full length human SMRT co-repressor, two isoforms of a mouse SMRT – a longer form, mouse SMRTα, and a shorter form, mouse SMRTβ, and an isoform of an insect (Drosophilia), SMRTER – as well as peptide portions of the SMRT co-repressors that can modulate transcriptional potential of a member of the nuclear receptor superfamily (nuclear receptor); to oligonucleotides that can hybridize specifically to such a polynucleotide; to vectors and to host cells containing such polynucleotides~~]. The invention also relates to polypeptide SMRT co-repressors encoded by [~~such~~] invention SMRT polynucleotides, and to peptide portions thereof that can modulate transcriptional potential of a nuclear receptor[~~; including peptide portions of a SMRT co-repressor that are not present in an N-CoR polypeptide~~]. In addition, the invention relates to chimeric molecules and to complexes containing a SMRT co-repressor or peptide portion thereof, to antibodies that specifically bind such compositions, and to methods for identifying an agent that modulates the repressor potential of a SMRT co-repressor. The invention also provides methods for identifying an agent that modulates a function of a SMRT co-repressor; for modulating the transcriptional potential of a nuclear receptor in a cell using the compositions of the

invention; and for identifying a molecule that interacts specifically with a SMRT co-repressor. --

In the claims:

Claims 1, 3, 5, 6, 9, 10, 12, 14, 15 and 23 have been amended as follows:

1. (Amended) An isolated polynucleotide encoding a member of a family of silencing mediators of retinoic acid receptor and thyroid hormone receptor **(SMRT)**, or an isoform or peptide portion thereof (**collectively, a** SMRT co-repressor), or an isolated polynucleotide complementary thereto**, wherein said SMRT co-repressor is capable of mediating the transcriptional silencing of at least one member of the steroid/thyroid hormone superfamily of receptors**.

3. (Amended) The polynucleotide of claim **1[2] and polynucleotides that hybridize thereto under stringent conditions**, wherein the SMRT co-repressor comprises a repression domain having

a) less than about 83% identity with a Sin3A interaction domain of N-CoR set forth as amino acids 255 to 312 of SEQ ID NO: 11;

b) less than about 57% identity with repression domain 1 of N-CoR set forth as amino acids 1 to 312 of SEQ ID NO: 11;

c) less than about 66% identity with a SANT domain of N-CoR set forth as amino acids 312 to 668 of SEQ ID NO: 11; or

d) less than about 30% identity with repression domain 2 of N-CoR set forth as amino acids 736 to 1031 of SEQ ID NO: 11[, ~~and polynucleotides that hybridize thereto under stringent conditions~~].

5. (Amended) A polynucleotide **acc rding t claim 1,** which hybridizes under stringent conditions with **SEQ ID NO:5** **[~~a polynucleotide according to claim 2~~]**.

6. (Amended) A polynucleotide **according to claim 1, which** **[~~that~~]** has at least 80% sequence identity with **SEQ ID NO:5** **[~~a polynucleotide according to claim 2~~]**.

9. (Amended) The polynucleotide of claim **8**[6], **wherein said polynucleotide encodes a polypeptide** having **substantially the same** **[~~an~~]** amino acid sequence as set forth in SEQ ID NO: 7 or conservative variations thereof.

10. (Amended) The polynucleotide of claim **8,** [4.] which has a nucleotide sequence **substantially the same** as set forth in SEQ ID NO: 6.

12. (Amended) The polynucleotide of claim 11, **wherein said polynucleotide encodes a polypeptide** having **substantially the same** **[~~an~~]** amino acid sequence as set forth in SEQ ID NO: 9 or conservative variations thereof.

14. (Amended) The polynucleotide of claim 1, comprising a nucleotide sequence selected from the group consisting of:

(a) nucleotides 1 to 3094 of SEQ ID NO: 4;

(b) nucleotides 1 to 3718 of SEQ ID NO: 6; **[~~and~~]**

(c) nucleotides 1 to 2801 of SEQ ID NO: 8 **and**

(d) **polynucleotides hybridizing under stringent conditions to (a), (b), or (c)**.

15. (Amended) A polynucleotide that **hybridizes** under stringent conditions with a polynucleotide according to claim 14, provided that the polynucleotide does not contain a sequence identical to SEQ ID NO: 11.

23. (Amended) An isolated oligonucleotide, comprising at least 15 nucleotides that can hybridize specifically to the polynucleotide of claim 1, but **neither** ~~[not]~~ to a polynucleotide encoding SEQ ID NO: 11 **nor** ~~[or]~~ to a polynucleotide encoding an amino acid sequence consisting of amino acids 1031 to 2517 of SEQ ID NO: 5.

Claim 2 has been cancelled without prejudice.

APPENDIX B –CLAIMS CURRENTLY UNDER CONSIDERATION

1. (Amended) An isolated polynucleotide encoding a member of a family of silencing mediators of retinoic acid receptor and thyroid hormone receptor (SMRT), or an isoform or peptide portion thereof (collectively, a SMRT co-repressor), or an isolated polynucleotide complementary thereto, wherein said SMRT co-repressor is capable of mediating the transcriptional silencing of at least one member of the steroid/thyroid hormone superfamily of receptors.

3. (Amended) The polynucleotide of claim 1 and polynucleotides that hybridize thereto under stringent conditions, wherein the SMRT co-repressor comprises a repression domain having

a) less than about 83% identity with a Sin3A interaction domain of N-CoR set forth as amino acids 255 to 312 of SEQ ID NO: 11;

b) less than about 57% identity with repression domain 1 of N-CoR set forth as amino acids 1 to 312 of SEQ ID NO: 11;

c) less than about 66% identity with a SANT domain of N-CoR set forth as amino acids 312 to 668 of SEQ ID NO: 11; or

d) less than about 30% identity with repression domain 2 of N-CoR set forth as amino acids 736 to 1031 of SEQ ID NO: 11.

4. The polynucleotide of claim 1, wherein the SMRT co-repressor is a human SMRT co-repressor having an amino acid sequence as set forth in SEQ ID NO: 5 or conservative variations thereof.

5. (Amended) A polynucleotide according to claim 1, which hybridizes under stringent conditions with SEQ ID NO:5.

6. (Amended) A polynucleotide according to claim 1, which has at least 80% sequence identity with SEQ ID NO:5.

7. The polynucleotide of claim 4, which has a nucleotide sequence as set forth in SEQ ID NO: 4, and conservative variations thereof.

8. The polynucleotide of claim 1, wherein the SMRT co-repressor is a mouse SMRTα isoform.

9. (Amended) The polynucleotide of claim 8, wherein said polynucleotide encodes a polypeptide having substantially the same amino acid sequence as set forth in SEQ ID NO: 7 or conservative variations thereof.

10. (Amended) The polynucleotide of claim 8, which has a nucleotide sequence substantially the same as set forth in SEQ ID NO: 6.

11. The polynucleotide of claim 1, wherein the SMRT co-repressor is a mouse SMRTβ isoform.

12. (Amended) The polynucleotide of claim 11, wherein said polynucleotide encodes a polypeptide having substantially the same amino acid sequence as set forth in SEQ ID NO: 9 or conservative variations thereof.

13. The polynucleotide of claim 11, which has a nucleotide sequence as set forth in SEQ ID NO: 8.

14. (Amended) The polynucleotide of claim 1, comprising a nucleotide sequence selected from the group consisting of:

(a) nucleotides 1 to 3094 of SEQ ID NO: 4;

(b) nucleotides 1 to 3718 of SEQ ID NO: 6;

(c) nucleotides 1 to 2801 of SEQ ID NO: 8 and

(d) polynucleotides hybridizing under stringent conditions to (a), (b), or (c).

15. (Amended) A polynucleotide that hybridizes under stringent conditions with a polynucleotide according to claim 14, provided that the polynucleotide does not contain a sequence identical to SEQ ID NO: 11.

16. A polynucleotide that has at least 80% sequence identity with a polynucleotide according to claim 14, provided that the polynucleotide does not contain a sequence identical to SEQ ID NO: 11.

17. A polynucleotide of claim 1, comprising a nucleotide sequence selected from the group consisting of:

nucleotides 1 to 8388 of SEQ ID NO: 6; and

nucleotides 1 to 7465 of SEQ ID NO: 8.

18. The polynucleotide of claim 1, comprising nucleotides 1 to 8561 of SEQ ID NO: 4.

19. The polynucleotide of claim 1, which is operably linked to a second nucleotide sequence.

20. The polynucleotide of claim 19, which encodes a fusion polypeptide comprising the SMRT co-repressor operably linked to a DNA binding domain of a transcription factor.

21. A vector comprising the polynucleotide of claim 1.

22. A host cell containing the polynucleotide of claim 1.

23. (Amended) An isolated oligonucleotide, comprising at least 15 nucleotides that can hybridize specifically to the polynucleotide of claim 1, but neither to a polynucleotide encoding SEQ ID NO: 11 nor to a polynucleotide encoding an amino acid sequence consisting of amino acids 1031 to 2517 of SEQ ID NO: 5.

24. The oligonucleotide of claim 23, wherein the polynucleotide encodes at least five contiguous amino acids of a sequence selected from the group consisting of:

amino acids 720 to 745 of SEQ ID NO: 5;

amino acids 716 to 742 of SEQ ID NO: 7; and

amino acids 497 to 523 of SEQ ID NO: 9.

25. The oligonucleotide of claim 23, which can hybridize specifically to a polynucleotide encoding SEQ ID NO: 5 or SEQ ID NO: 7, but not to a polynucleotide encoding SEQ ID NO: 9.